Supplementary Information

Modular Nucleic Acid Assembled p/MHC Microarrays for Multiplexed Sorting of Antigen-Specific T Cells

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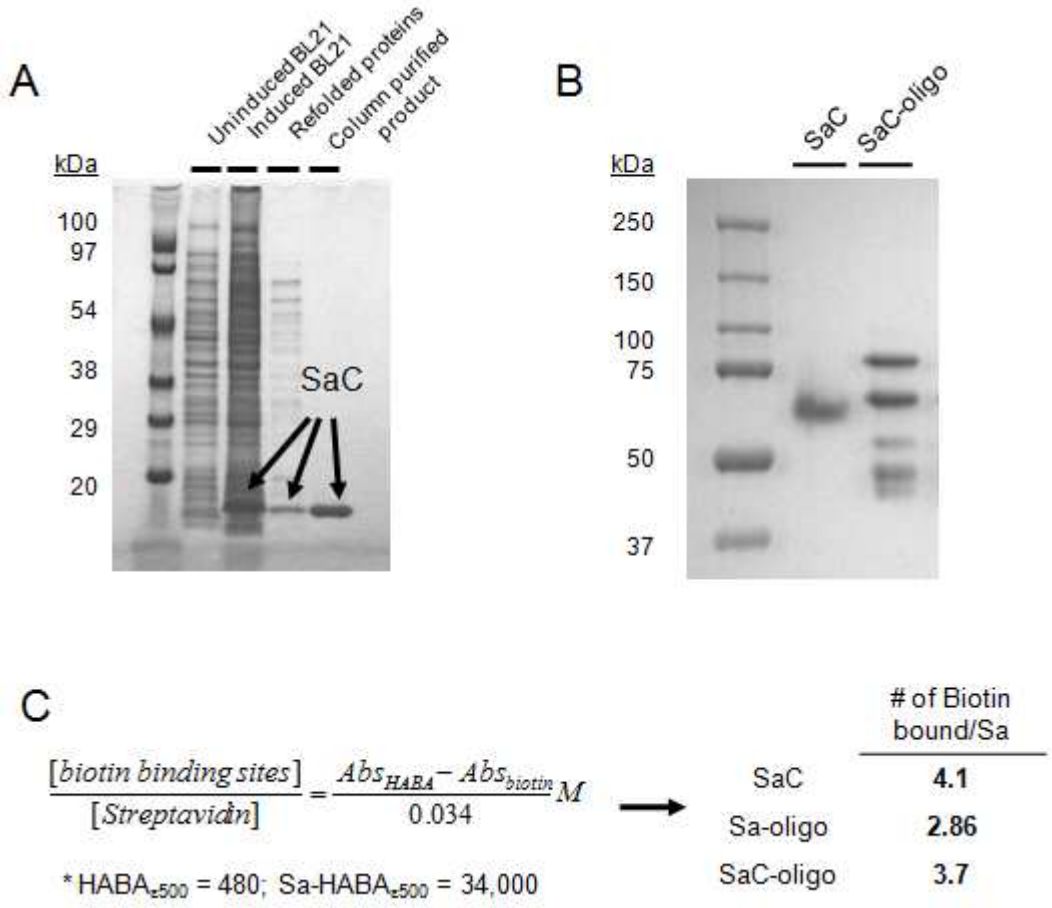

Figure S1. An engineered variant of streptavidin expressing C-terminal cysteine residues has superior biotin binding capacity compared to native streptavidin post conjugation with ssDNA. (A) The various stages of SAC expression, refolding, and purification were analyzed on a denaturing PAGE gel. The molecular weight of a SAC monomer is ~12kDa. (B) A gel mobility shift assay to verify the formation of ssDNA-SAC conjugates. Individual bands representing SAC-oligo conjugates differing by one DNA strand can be resolved. Lower order SAC-oligo conjugates (1-2 oligos per protein) run "lighter" when compared to unmodified SAC because of the difference in charge/mass density of nucleic acids. Higher order SAC-oligo conjugates corresponding to 3-4 DNA strands per SA were favored. (C) The molecule 2-(4'-Hydroxyazobenzene) benzoic acid (HABA) was used to determine the molar ratio of association of biotin to SA. Native SA-oligo conjugates bound ~2.9 moles of biotin per mole of SA, a significant decline when compared to the 4:1 ratio of unmodified SAC. SAC-oligo conjugates maintained near optimum binding capacity (3.7:1).

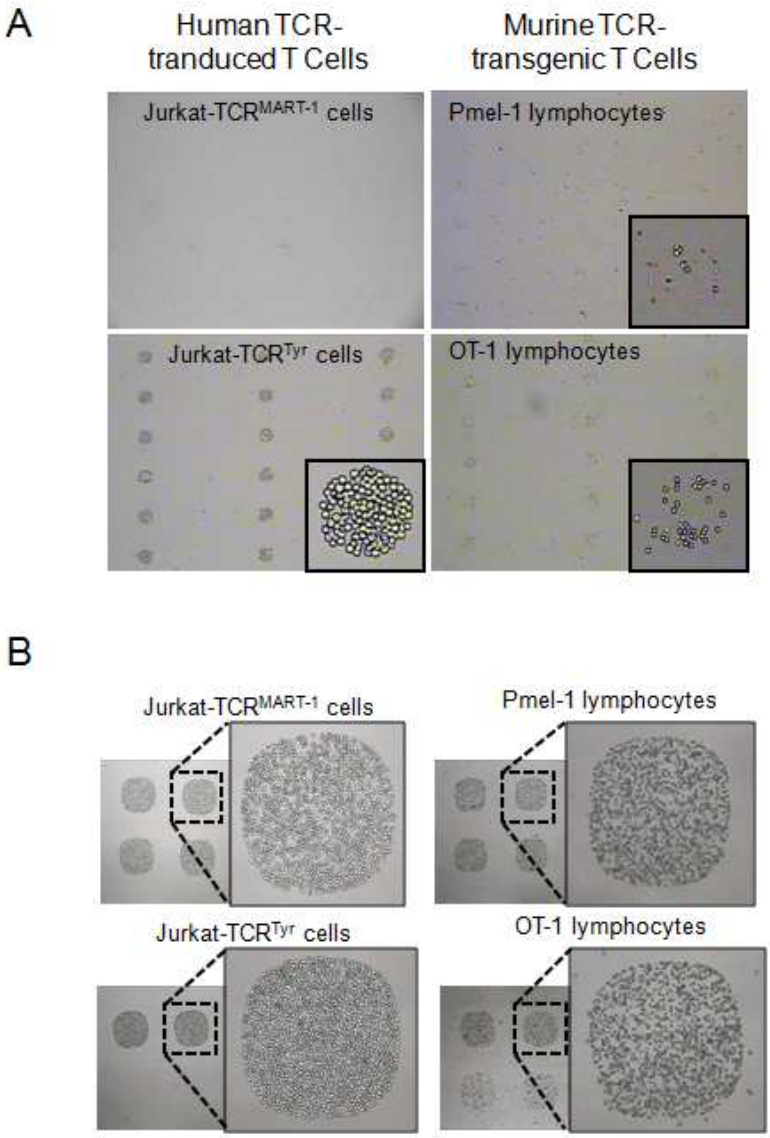

Figure S2. T cell capture efficiency is optimal when utilizing ssDNA-SAC conjugates to generate NACS p/MHC tetramers (A) ssDNA-p/MHC constructs derived from native SA were used to sort 4 different human/murine transgenic T cell populations. The T cell capture efficiencies were highly varied amongst the four T cell populations. (B) ssDNA-p/MHC tetramers derived from ssDNA-SAC conjugates were used to sort the four T cell populations. The resulting cell capture efficiencies were markedly improved over native SA-oligo conjugates, demonstrating that SAC is necessary for the production of high affinity ssDNA-p/MHC tetramers.

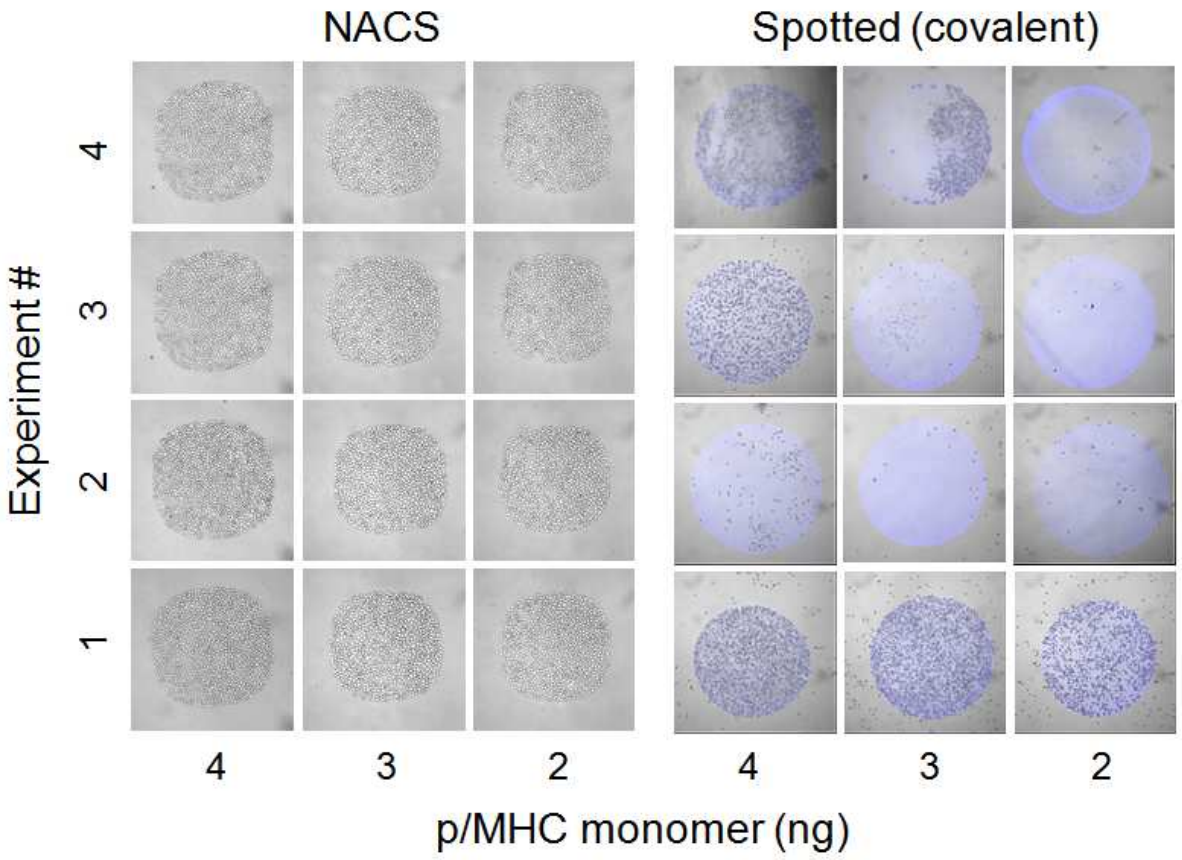

Figure S3. Comparison of the performance of p/MHC arrays produced by NACs and by spotting The consistency and robustness of T cell immobilization with NACS is evident when compared directly with spotted arrays, which suffers from significant levels of inter-spot, intra-spot, and inter-experimental heterogeneity. Each row represents a separate experiment performed on a different slide.

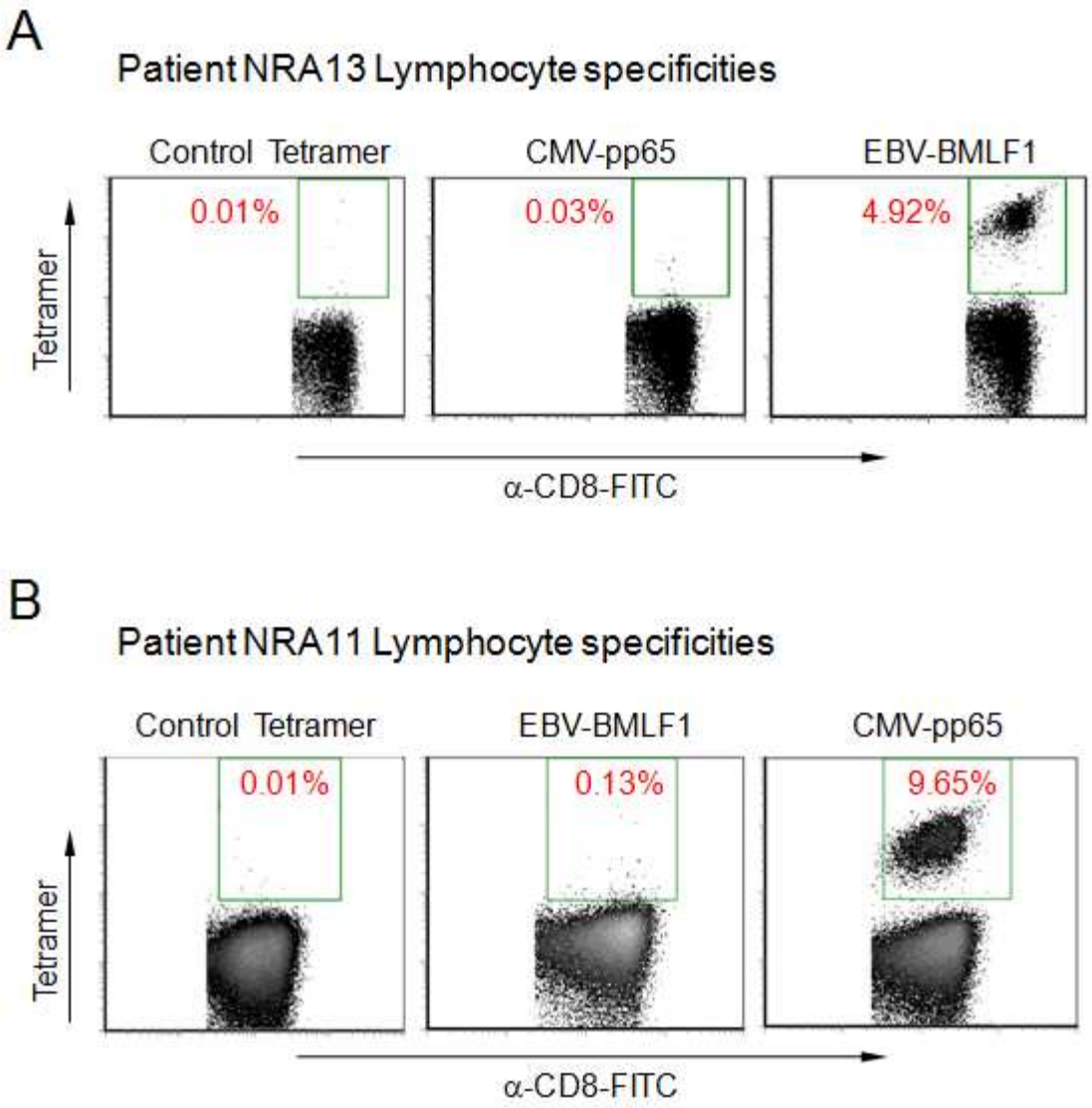

Figure S4. The T cell specificities of PBMCs from patients NRA11 and NRA13 (A) Lymphocytes isolated from NRA13 contained significant levels of EBV specific T cells (4.9%) with minimal CMV specific T cells. (B) Lymphocytes isolated from NRA11 contained high levels of CMV specific T cells (9%) with a low population of EBV-specific cells (0.12%). Leukaphersis fractions from both patients were kept as frozen aliquots before thawing, CD8+ enrichment and NACS sorting.

Table 1. Orthogonal DNA sequences for spatial encoding of p/MHC tetramers

Name	Sequence*
A	5' - AAA AAA AAA AAA AAT CCT GGA GCT AAG TCC GTA AAA AAA AAA AAT CCT GGA GCT AAG TCC GTA AAA AAA AAA AAA A
A'	5' - NH_2- AAA AAA AAA ATA CGG ACT TAG CTC CAG GAT
B	5' - AAA AAA AAA AAA AGC CTC ATT GAA TCA TGC CTA AAA AAA AAA AGC CTC ATT GAA TCA TGC CTA AAA AAA AAA AAA A
B'	5' - NH_2- AAA AAA AAA ATA GGC ATG ATT CAA TGA GGC
C	5' - AAA AAA AAA AAA AGC ACT CGT CTA CTA TCG CTA AAA AAA AAA AGC ACT CGT CTA CTA TCG CTA AAA AAA AAA AAA A
C'	5' - NH_2- AAA AAA AAA ATA GCG ATA GTA GAC GAG TGC
A_{EcoRI}	5' - AAA AAA AAA AAA GAG CTA AGT CCG TAG AAT TCA AAA AAA AAA GAG CTA AGT CCG TAG AAT TCA AAA AAA AAA AAA
A_{EcoRI}'	5' - NH_2 – AAA AAA AAA AGA ATT CTA CGG ACT TAG CTC CAG GAT
B_{BamHI}	5' - AAA AAA AAA AAA TTG AAT CAT GCC TAG GAT CCA AAA AAA AAA TTG AAT CAT GCC TAG GAT CCA AAA AAA AAA AAA
B_{BamHI}'	5'- NH_2 – AAA AAA AAA AGG ATC CTA GGC ATG ATT CAA TGA GGC

* All sequences to be conjugated to SAC (A', B', C', A_{EcoRI}', and B_{BamHI}') were designed with a polyA linker followed by a 20mer hybridization region. The 5' amine is required for the attachment of the hetero-bifunctional maleimide derivative MHPH. Sequences printed on glass substrates (A, B, C, A_{EcoRI}, and B_{BamHI}) were designed with two hybridization regions separated by polyAs. This was designed to facilitate electrostatic adsorption to amine glass substrates.

Full References

39) Dudley, M.E.; Wunderlich, J.R.; Robbins, P.F.; Yang, J.C.; Hwu, P.; Schwartzentruber, D.J.; Topalian, S.L.; Sherry, R.; Restifo, N.P.; Hubicki, A.M.; Robinson, M.R.; Raffeld, M.; Duray, P.; Seipp, C.A.; Rogers-Freezer, L.; Morton, K.E.; Mavroukakis, S.A.; White, D.E.; Rosenberg, S.A. Science 2002, **2002**, *298*, 850-854.